European Review for Medical and Pharmacological Sciences 2012; 16: 1925-1933

From molecular imaging in preclinical/clinical oncology to theranostic applications in targeted tumor therapy

C. ALBERTI

L.D. of Surgical Semeiotics, Parma, Italy

Abstract. – BACKGROUND: The impact of constantly developing molecular sciences on the various imaging modalities (particularly nuclear, magnetic resonance-based, optical techniques) has produced the beginning of a new complex science – the molecular imaging – that, through the exploitation of specific molecule-probes instead of nonspecific conventional contrast materials, is aimed at the characterization of the tumor-related molecular abnormalities, to adopt innovative targeted therapeutic measures, even at the genetic level.

OBJECTIVES: Aim for this review is to focus on recent significant accomplishments of different molecular imaging modalities moreover outlining the challenges of current theranostic developments.

EMERGING KNOWLEDGES: The spatial resolution of almost all imaging techniques is more and more increasing, so that some experimental in vivo imaging modalities can allow an extremely detailed three-dimensional resolution. From the constant developments of molecular biology it follows that, instead of relatively gross conventional diagnostic criteria on malignancies (anatomic location and size, surrounding tissue involvement, distant spread), more specific molecular imaging parameters might be adopted – such as tumor cell kinetics, genetic alterations, variety of involved growth factors – to reach, by innovative targeted drugs and biological agents, therapeutic effects at the molecular level. In animal models – particularly in cancer xenografts – the molecular imaging, through the resort to SIAFS (small animal imaging facilities), allows *in vivo* thorough investigations on the tumor development-related mechanisms, furthermore improving the research on pharmacokinetics and pharmacodynamics of newly developed drugs.

CONCLUSIONS: Current applications of molecular imaging are due to its capability of both *in vivo* identifying tumor early molecular abnormalities and monitoring personalized therapies. Foreseeably the research advances will tremendously expand in the near future, particularly considering that simultaneous both imaging- and therapy implications of the theranostics can improve, to the highest degree, the potential of molecular imaging.

Key Words:
Nuclear imaging, MRI, Optical imaging, SAIFS, Cancer xenografts, Tissue engineered models, Nanotechnology.

Introduction

As result of an intersection of molecular biology, bioinformatics and *in vivo* imaging, the *molecular imaging* allows the detection of specific molecular targets, from those pertaining to gene expression to that regarding protein products and their specific functions[1,2].

Among different diagnostic imaging modalities – computed tomography (CT), planar scintigraphy, positron emission tomography (PET), single photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), ultrasound (US) and optical imaging (OI) – particularly PET, SPECT, MRI, and OI are currently used for noninvasive molecular imaging, given their capability to exactly identify molecular targets inside living organisms[3]. Such above imaging modalities differ mainly in these following features: spatial/temporal resolution, sensitivity, tissue penetration depth, imaging energy expended (Table I).

What's more, the nanotechnologies, by the use of nanoparticles as multi-functional/multi-stage nanovectors of both molecular imaging nanotools and therapeutic nanoagents, allow them to avoid various tissue-related immune reactions and cross cell-membrane barriers, so they might directly interact, at the same size regime, with intracell select targets as single proteins and single genes.

Positron Emission Tomography (PET)

PET allows the *in vivo* three-dimensional visualization of molecular targets and related metabolic-functional processes, by the use of pharmaceuticals labelled with *positron-emitting radionu-*

Corresponding Author: Contardo Alberti, MD; e-mail: eneide94@gmail.com

Table I. Different molecular imaging modalities: advantages and disadvantages.

Energy source Imaging technology	Favourable properties	Drawbacks	Probe typology
• Nuclear (ionizing radiations)			
– PET	High sensitiviy (≈ 10^{-13} mol/L) Metabolic-dynamic informations	Low spatial resolution (1÷2 mm)	Positron-emitting radionuclides
– SPECT	High sensitiviy (≈ 10^{-14} mol/L) Metabolic-dynamic informations	Low spatial resolution (1÷2 mm)	γ-emitting radionuclides
• RM-based (magnetic field+radiofrequencies)	High spatial resolution (50 μm) Metabolic-dynamic informations	Low sensitivity (enhanced by selected atom hyperpolarization)	Free MR, paramagnetic or superparamagnetic contrast materials, USPIO
• Optical (nonionizing radiations, in the field of light wave-lengths or near-infrared, NIR)	Capability of differentiating a variety of probes	Low tissue penetration, improved by resort to NIR	Bioluminescent or fluorescent materials

clides, produced by particle accelerators (cyclotrons). Positrons (β^+) annihilate through an impact with nearby microenvironmental electrons (e^-), so emitting two 0.511 MeV phothons that, thrown 180 degrees apart in opposite directions, are then detected, in coincidence manner, by scintillation crystals (bismuth germanate, lutetium or gadolinium orthosilicate, barium fluoride, etc), which are full-ring arranged and coupled with photomultipliers. The images, resulting from reconstruction algorithm-based post-processing, reproduce the spatial biodistribution of positron-emitting tracers (Table I).

First and foremost advantage of PET is the very high sensitivity, given the ability to *in vivo* measure concentrations of a radiotracer, in a well-defined area, in the order of picomolars (10^{-11} ÷ 10^{-15} mol/L). The spatial resolution, instead, by itself low, may be improved by a suitable image reconstruction post-processing. Several positron emitters unfortunately have an extremely low half-life (e.g., 20.38 min for ^{11}C, 9.96 min for ^{13}N, 2.04 min for ^{15}O), therefore, requiring on-site cyclotron together with neighbouring radiopharmacy for the synthesis of radiopharmaceuticals. Others, indeed, are endowed with higher half-life, such as ^{94m}Tc (52 min), ^{124}I (4.2 days), ^{68}Ga (67.6 min), ^{64}Cu (12.700 hours).

As far as the *tumor molecular imaging* is concerned, PET can properly target either some metabolic-functional cell growth-related peculiarities, such as enhanced glucose metabolism and thymidine-kinase activity, or cell death-associated dynamic events or even various ligands/cell-receptors interactions. Indeed, the most commonly PET oncological studies concern the glucose metabolism by the use of glucose analog 2-deoxyglucose labelled with ^{18}F (half-life = 109.8 min). This radiotracer, [^{18}F]FDG, once inside the cells, is phosphorylated, like native glucose, by hexokinase-2, to [^{18}F]FDG-6-P, that, because of presence of a fuorine-atom instead of the hydroxyl group in position 2, is unable to go on glycolytic cascade, hence gathering inside the cells and, therefore, enhancing the visualization of radiotracer-targeted site[4].

Unfortunately, [^{18}F]FDG-PET suffers from lack of specificity in tumor studies because such radiotracer is also uptaken from inflammatory cells, so that the use of specific tumor growth molecules targeting radiopharmaceuticals – such as [^{18}F]-thymidine, [^{11}C]-thymidine, [^{11}C]-methionine, [^{11}C]- or [^{18}F] choline – results more advantageous[1].

Interestingly, PET molecular imaging is one of leading tools to show proteomic changes – particularly the hypoxia-inducible factor-1 (HIF) expression in cancer cells, thus allowing them to be resistant to radio-/chemiotherapy under hypoxic conditions – by using suitable radiopharmaceuticals such as [^{18}F]-FETNIM (fluoroerythronitroimidazole), [^{18}F]-FMISO (fluoromisonidazole), [^{18}F]-FAZA (fluoroazomycinarabinoside), [^{62}Cu]-ATSM (diacetil-methylthiosemicarbazone)[2].

As for PET with radiolabelled analogs of choline – metabolic precursor of membrane phospholipids – [^{11}C]-choline-PET, though exhibiting very high sensitivity and specificity, even higher than MR-spectroscopy, nevertheless, has the drawback of too short half-life of ^{11}Carbon, its use remaining restricted to PET centres

equipped with an on-site cyclotron[5-7]. Thankfully, [^{11}C]-choline-PET-like results may be achieved by the use of [^{18}F]-choline-PET, as ^{18}Fluorine, given its longer half-live, proves to be available to any PET unit, though unprovided with cyclotron. Anyhow radiolabelled, the choline, besides its higher tumor-targeting specificity *versus* [^{18}F]FDG, is negligibly excreted in urine, thus not impairing the image interpretation of either bladder or prostate tumors[8-12].

Other PET agents, such as radiolabelled aminoacids or nucleotides, can play an important role in tumor molecular imaging, given their capability to show either protein or nucleic acid synthesis pathway[8]. Among the radiolabelled aminoacids, fusion-imaging of ^{11}C-methionine-PET/MRI may 3-dimensionally localize, with high specificity and without inflammatory interferences, the increased protein synthesis from tumors[13,14].

In the field of pure *neuroendocrine tumors/ neuroendocrine differentiation of tumors*, the reliability of PET-molecular imaging by use of aforesaid radiofarmaceuticals is very poor because of nonsignificant hypermetabolic/hyperproliferative behaviour of such tumors while useful resulting, instead, the resort to a specifically neuroendocrine tissue-uptaken radiotracer [^{11}C]-5-hydroxytryptophan – as precursor of 5-hydroxytriptamine, serotonin – that is able to supply specific informations on the primary site of neuroendocrine tumors and their metastatic spread[15].

Single Photon Emission Computed Tomography (SPECT)

As opposed to positron emitting agents, the radionuclides used in SPECT – such as 99^{m} Technetium (^{99m}Tc), 111 Indium (^{111}In), 123 Iodine (^{123}I), 133 Xenon (^{133}Xe), 201 Thallium (^{201}Tl) – emit γ-rays and, compared to positron emitters, have the advantage of relatively longer half-life, thus not requiring on-site cyclotron for their production (Figure 1).

The SPECT uses a large number of γ-emitting radiopharmaceuticals, from small molecules to antibodies (imuno-SPECT), some of which widely used in clinical practice. Such molecular imaging technique allows to monitor different functional-metabolic processes, quantify various receptors density, define particular cell events such as apoptosis, moreover reaching, when integrated with CT (fusion-imaging), reliable morphological findings, so overcoming its intrinsic limits as for spatial resolution[16].

In the field of oncology, because of high requirement of aminoacids from uncontrolled growth of cancer cells and, hence, a tumoral upregulation of aminoacid-transporters expression, such cell-carriers represent an interesting target for tumor molecular imaging, so that several radiolabelled both natural and synthetic aminoacid-based probes may be used for SPECT-, besides PET-, tumor molecular imaging[17].

As for *immuno-SPECT*, murine monoclonal antibodies directed against specific cancer molecular targets (e.g., regarding the prostate carcinoma, PSMA, prostate-specific membrane antigen, over-expressed by malignant prostate epithelial cells), radiolabelled with ^{111}In-pentetide, are specifically uptaken by primary tumor focus – moreover, also showing a possible local recurrence – together with by involved lymph nodes and distant metastases[8,18-20].

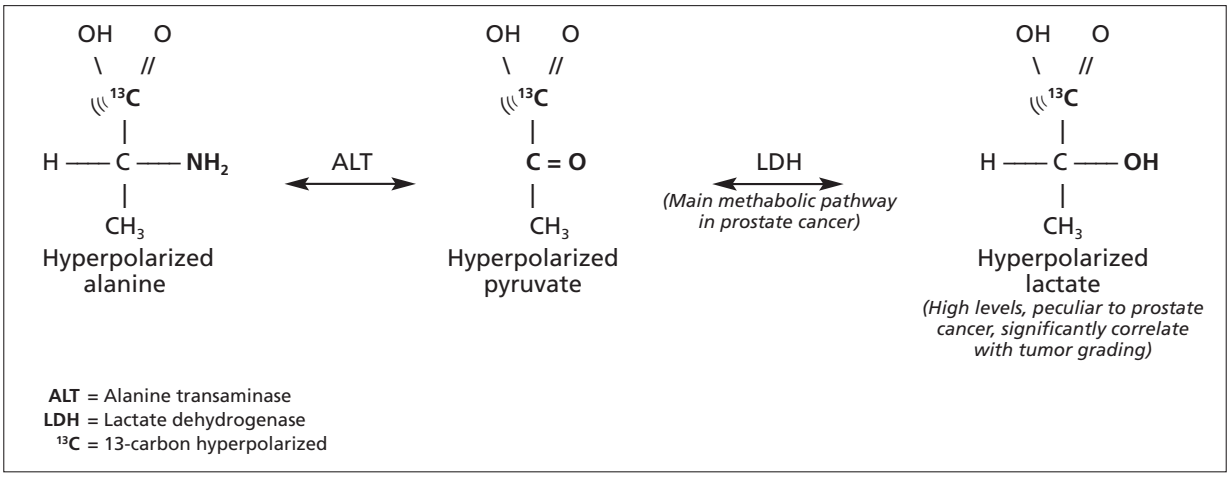

Figure 1. Hyperpolarized ^{13}C pyruvate and its metabolic products, in 3D-MRSI (acc. to 22).

Magnetic Resonance Imaging

MRI, as a versatile molecular imaging modality, can show metabolic-functional processes together with producing morphological images with very high resolution. Unfortunately, MRI has a lower sensitivity ($10^{-3} \div 10^{-5}$ mol/L) compared with other imaging techniques, what's due to non-significant difference of various atoms energy status. Hence, the opportunity of improving MR sensitivity by either increasing the magnetic field or hyperpolarizing selected atoms through dynamic nuclear polarization (DNP) or optical pumping technique, to reach a highly significant enhancement of MR signals from different atoms[21,22]. Magnetic resonance spectroscopy (MRS) can non-invasively provide usefull informations reflecting biochemical changes within different pathological processes – particularly in tumors – to complement MRI morphological findings.

Hyperpolarized ^{13}C-based three-dimensional molecular MR spectroscopic imaging (3D-MRSI) may be utilized to detect and characterize a variety of tumors – particularly the prostate carcinoma – by differentiating hyperpolarized ^{13}C-pyruvate from its metabolic derivatives ^{13}C-alanine and ^{13}C-lactate, the last product resulting especially increased given the high tumoral LDH (lactate-dehydrogenase) activity[21,22] (Figure 1). Consequently, hyperpolarized ^{13}C-bicarbonate-based MRI can show a tumor average interstitial pH significantly lower compared to surrounding normal tissue[23].

Even though without the resort to previous administration of ^{13}C-choline, the three-dimensional-chemical shift imaging-magnetic resonance spectroscopy (3D-CSI-MRS) allows the detection of malignancy-related both high levels of choline and low concentrations of citrate compared with those shown in normal tissues[24].

To differentiate malignancies from fibrotic processes (e.g., hepatocarcinoma from cirrhosis), the molecular MRI, by resorting to intravenously administered type I-collagen-targeted probe EP-3533, may noninvasively recognize, within the fibrous tissue, a significant accumulation of extracellular matrix proteins, particularly of type I-collagen-derived hydroxyproline[25].

Recently, *magnetic liposomes* – phospholipid vesicles encapsulating magnetic or paramagnetic ultrasmall particles such as ultrasmall superparamagnetic iron oxides ($USPIO_S$) – have been used as MRI contrast materials whose surface may be advantageously provided, compared to free magnetic particles, with different functional groups that can *in vivo* target specific cell-molecules[26,27]. Paramagnetic lanthanide (Gd-based) complexes, endowed with anisotropic electronic configuration, can induce strong effects on MR frequency of the coupled spins dipolarity[28].

Furthermore, multimodal contrast materials allow their simultaneous detection by more than one imaging modality, so that, by attaching an optical probe to a MR contrast material, it is possible, after the whole body *in vivo* molecular RM imaging-research, validate RM findings by histological studies[50].

Optical Imaging (OI)

Different approaches of optical imaging (OI), especially depending upon either bioluminescence or fluorescence, besides applications in cell biology (fluorescence microscopy), allow *in vivo* surface molecular imaging studies, even recently developed with the optical coherence tomography[29] (Table I).

The OI "beacons", with high molecular specificity, may suitably *in vivo* provide images of enzyme activity together with metabolic pathways, and visualize both tumor protein (e.g., cathepsin D, a lysosomal glycoprotein)- and nucleic acid targets.

Fluorescence OI-related advantages are both the high sensitivity and the specificity as well as the involvement of low-energy electromagnetic radiations (nonionizing radiations) in the field of visible light spectrum and near-infrared (NIR). The disadvantage of OI is the lack of penetration dept, especially at visible wave-lenghts, depending on tissue-related light absorption and scattering. Because of considerably lower tissue absorption coefficient in the region of NIR ($0.7 \div 3$ μm), the most commonly molecular OI studies are carried-out resorting to NIR-fluorescent probes.

Fluorochromes or fluorophores, like cell/tissue natural chromophores – such as β-carotene, lycopene, anthocyanins, metal-complexes as hemoglobin/hemocyanin/chlorophyl – are fluorescent chemical compounds which absorb light energy of a specific wavelength and re-emit that at a longer wavelength with a lower energy. Besides the fluoroscein, an isothiocyanate, other fluorophores are, among nonprotein compounds, rhodomine, cyanine, coumarin, acridine-orange, while, among the protein-based ones, GFP (green fluorescent protein), YFP (yellow fluorescent protein) and RFD (red fluorescent protein), all such types generally bounded, as markers (dyes, tags, reporters, labels), to macromolecules (nucleic acids, peptides, antibodies). Particularly, GFP has been proposed as

tag not only for studies of cellular processes and various drug pharmacodynamics/pharmacokynetics but also for *in vivo* optical imaging[30]. Moreover, RDG (arginine, glycine-aspartate peptide), covalently conjugated with an organic dye (e.g., Cy 5.5) and bound to human serum albumin, allows to *in vivo* show high tumor accumulation, given the specific binding of RDG peptide with αVβ3 integrin receptor protein, that is highly expressed in tumoral tissues[31]. Just regarding it, RDG-Cy 5.5 dye complex-based molecular OI can reliably show, in animal models, the growth course in *cancer xenografts*, together with their response to therapy[33]. Also 2-deoxy-glucose, besides its use in FDG-PET, may be labelled, for *in vivo* OI studies in cancer xenografts, with a NIR fluorophore (e.g., IR-dye 800CW)[34].

In large animals approaching the human body size, NIR fluorescent quantum dots (QD_S), endowed with semiconductive properties together with peculiar bright emission, have been used, as tumor targeting agents, to map sentinel lymph node[32] but, because of their nano-size-dependent insufficient renal elimination and, therefore, their potential nephrotoxicity – apart from novel dendron-coated QD_S, provided with suitable both size and renal clearance – are clinically unadvisable[31,33]. Instead, for such purpose, other OI contrast materials may be used, such as either heptamethine-indocyanine-based-fluorophore or, still better, the fluorescent human serum albumin given its high performance to penetrate lymphatics and reach sentinel lymph nodes where it is specifically retained[32].

High sensitivity of bioluminescence imaging, involving luciferase enzyme – that, in presence of ATP (adenosine triphosphate), magnesium and oxygen, produces light emission – may be used, in animal models, to monitor tumor onset, progression and response to various treatment strategies[35,36]. Interestingly, in tumor animal models, a transfected and adequately silenced luciferase gene reporter, may be activated, so becoming noninvasively detectable in bioluminescence molecular imaging as soon as caspase-3 enzyme is produced, particularly under pro-apoptotic agent-mediated conditions (proapoptotic drugs; TRAIL, TNF-α-related apoptosis-inducing ligand), within apoptotic cells[36].

What's more, effects of chemoteraphy on cancer animal models (e.,g., cisplatin on lung cancer animal model) have been accurately studied via noninvasive luciferase reporter gene-related bioluminescent molecular OI focused on p53 activity[37].

Molecular Imaging to Monitor Gene Therapy

Aim of gene therapy is to achieve permanent changes into gene-constitution through various mechanisms, among which corrective gene repair, gene suppression or gene addition. More and more taken into consideration also the antisense therapy – administration of short oligonucleotides to block gene expression – and the use of short inferfering RNA_S ($siRNA_S$) to target specific RNA messengers ($mRNA_S$) to arrest genetic information pathway.

Gene oncotherapy includes both the immunotherapeutic strategies to deliver genes encoding for cytokines and the *suicide gene therapy*, an antiproliferative-cytoreductive treatment modality based on the use of so-called *suicide genes* that are able to induce sensitivity of cells to nontoxic prodrugs. So, e.g., the herpes simplex virus1-derived thymidine kinase (HSV1-TK) suicide gene can convert, by phosphorilation, nontoxic Ganciclovir (arabinosine-furenosyl-uracil) into toxic Ganciclovir-triphosphate, thus reaching antiproliferative effects[38,39]. What's more, instead of a necrotic tumor cell death, the apoptosis may be obtained through pro-apoptotic gene transfer, e.g., by using dominant negative mutant of cyclin G- or p53 gene[38].

In vivo molecular imaging of gene expression aims to detect successfully produced gene-dependent specific proteins. The most commonly used molecular imaging technique to monitor gene therapy is PET, by using, as a case in point, [^{18}F]-F-ganciclovir or penciclovir reporter probe, showing viral thymidine kinase gene reporter expression[40,41]. Furthermore, β-galactosidase genereporter may be indirectly identified on the basis of its action on galactosylated chelator substrates, whose cleavage-derived galactose residues are, in turn, detected, on the basis of relaxivity changes, by paramagnetic gadolinium (Gd) probe-based MRI[40]. What's more, bioluminescent molecular OI, via noninvasive luciferase reporter gene, can, in cancer animal models, show p53 activity changes under chemotherapy, so representing a useful tool to *in vivo* study, besides in cell culture, the effects of anticancer drugs[37].

Molecular Imaging in Small Animal Models and Cancer Xenografts

The need to carry out longitudinal research studies in small animal models reproducing human diseases (genetic disorders, malignancies, etc) – by repeatedly studying the same animal to

monitor the disease course and the response to potential new pharmaceuticals – has recently led to develop a variety of molecular imaging tools, that might be suitable for small animals (SAIF, small animal imaging facilities), particularly micro-PET, micro-SPECT, micro-MRI and micro (biolumiscence or fluorescence)-OI[16]. Each among such *preclinical* imaging modalities aims to optimize the research directed to identify metabolic-functional processes, track drug-kinetics and -dynamics, quantify receptor-protein expression, monitor gene-therapy. Recently, in animal models, molecular imaging has been applied to study patient-derived *cancer xenografts*, so *in vivo* providing informations on both different gene expression and molecular profiling, receptorial protein density, tumor growth, cell apoptosis, together with responses to anticancer treatments[42-45]. As for evidence regarding it, αVβ3-integrin-positive melanoma xenografts may be suitably identified and studied by RDG-labelled NIR fluorophore[31,33].

Intriguingly, the molecular imaging research, rather than on cancer xenografts in animal models, could be accomplished on *cancer xenografts* implanted on to *three-dimensional tissue-engineered* organ-like structures (liver, skin, etc), supplied by a biological vascularized scaffold generated from decellularized animal organ (e.g., segment of porcine small bowel) with preserving vascular network within extracellular matrix. Bioreactors can sustain physiological tissue conditions by providing 3D-engineered organ like structure, together with implanted patient-derived tumor, with proper regulatory cell growth factors. Thus, without resorting to experimental animals, the molecular imaging could provide, in such cancer grafts, usefull informations – like those in animal models – on gene expression profile and different protein production[46-49].

Theranostic Applications in Targeted Tumor Therapy

Quite recently, in the field of *theranostics* (fusion-term of θεραπεια, *therapy*, with γνωσις, *dia-gnosis*) – a new technology that concurrently combines molecular imaging and personalized therapeutic capability within a single platform – multi-dye silica nanoparticles, composed of both fluorescent (heptamethine cyanine) and photothermal (metallo-naphtalocyanine) agents within a single nanoconstruct, have been proposed to allow tumor NIR fluorescence visualization together with providing a modality of simultaneous photothermal ablation – tumor necrosis by NIR-laser excitation-induced increase in temperature – in oncological surgery[50,51]. With full particulars, phtalocyanine-based chromophores, given their potential as high-affinity guanine-quadruplex (DNA belonging-guanine tetrads) ligands – that in telomeric DNA inhibit telomerase activity – may represent promising anticancer drugs[51].

Moreover, photosensitive nanoparticles (gold-nanoshells; goldrods, $AuNP_S$), as provided with photothermal property of both strongly adsorbing e-m bands in NIR range, particularly NIR laser beams, and converting the adsorbed energy to intense heat, may be incorporated into a novel mesoporous silica-made up material whose surface has been functionalized with an aptamer targeting DNA agent (or an anticancer aptamer AS1411). Upon application to NIR laser beam, the phototermal effect of $AuNP_S$ induces *tout court* rise in local temperature allowing the controlled release of the anticancer agent in cancer cells, so that integrate into one multifunctional platform both molecular imaging and photothermaltherapy (burning of malignant cells) with, in addition, a drug anticancer therapy[52,53].

What's more, multifunctional ethylene/acrylate co-polymer-based vesicles, endowed with USPIO together with DOX-HCl anticancer drug, can exhibit both ultrasensitivity for MRI and targeted anti-tumor drug effectiveness[54].

Interestingly, in the field of theranostics, strong developments are more and more emerging from the concurrently combination, within a single platform of molecular imaging specific PET probes with appropriate radionuclide-based personalized radiometabolic effects-reaching therapeuticals[55].

Among the multimodal theranostic applications, the molecular imaging-guided findings may play an important role, besides through showing the malignancy-related molecular peculiarities, also by targeting and monitoring tumor therapy[56-57].

Conclusions on Current Research and New Directions

Molecular imaging has emerged from joining molecular biology to different *in vivo* imaging technologies, so allowing to *in vivo* visualize molecular processes and various cell events and obtain imaging acquisitions on gene expression and genetic alterations[58-60]. In addition, molecular imaging may provide, in preclinical studies, useful data on pharmacokinetics and pharmacodynamics of newly developed drugs, particularly regarding the innovative targeted therapies. On this subject, in the last two decades, different small

animal imaging facilities ($SAIF_S$) have been developed – such as micro-PET, -SPECT, -MRI, -OI – together with widening their applications to researh on *cancer xenografts* in animal models, which could be favourably replaced – at least as for studies on gene expression and tumor protein products – by those on cancer xenografts in tissue engineered models[16,47,48,61].

Intriguing developments of other molecular imaging techniques are today in progress, including those based on radiofrequency- conditioned biological behaviour, wherein a selected biomolecule (protein, peptide, nucleic acid), covalently linked to a metal nanocrystal-based antenna, becomes responsive to external specific radiofrequency signals, to that to be *in vivo* precisely located through molecular imaging and timely guided[46,62]. Among such techniques the MENS, micro-electronic mechanical system, allowing to directly show cell activity also in living animals, is more and more taken into consideration[63].

Recent advances in nanotechnology are opening new thorough knowledges on interactions between different nanobiomaterials and biosystems, so improving both molecular imaging and therapeutic measures.

Moreover, molecular imaging, by offering the advantage of a very early diagnosis, allows *minimally invasive surgical approaches*, such as, expecially, image-guided tumor ablation targeted endoscopic procedures[64]. Indeed, the most valuable modalities to treat the malignancies lie in both early detecting- and, subsequently, quite resecting them. With this respect, NIR-fluorescent probes, such as tumor-specific NIR emitting quantum dot-bioconjugates, when intravenously injected, can intraoperatively produce a definite tumor-targeted imaging, preliminarily to prompt tumor resection that can be performed via NIR guidance resorting to the Fluobeam-700 technique[65].

Very interestingly, simultaneous imaging-and therapy implications of the theranostics can improve, to the highest level, the potential of molecular imaging.

References

1) De Saint-Hubert M, Bauwens M, Verbruggen A, Mottaghy FM. Apoptosis imaging to monitor cancer therapy: the read to fast treatment evaluation? Curr Pharm Biotechnol 2012; 13: 571-583.
2) Kurihara H, Honda N, Kono Y, Aray Y. Radiolabelled agents for PET imaging of tumor hypoxia. Curr Med Chem 2012; 19: 3282-3289.
3) Grassi R, Lagalla R, Rotondo A. Genomics, proteomics, MEMS and SAIF: which role for diagnostic imaging? Radiol Med 2008; 113: 775-778.
4) Giorgetti A, Volterrani D, Mariani G. Clinical oncological applications of PET using Fluorine-18-Fluoro-2-deoxy-D-glucose. Radiol Med 2002; 103: 293-318.
5) Jana S, Blaufox MD. Nuclear medicine studies of the prostate, testes and bladder. Semin Nucl Med 2006; 36: 51-72.
6) Langsteger W, Heinisch M, Fogelman I. The role of fluorodeoxyglucose, 18F-dehydroxyphenilalanine, 18F-choline and 18F-fluoride in bone imaging with enphasis on prostate and breast. Semin Nucl Med 2006; 36: 73-92.
7) Pelosi E, Arena V, Skanjeti A, Pirro V, Douroukas A, Pupi A, Mancini M. Role of whole-body 18F-choline PET/CT in disease detection in patients with biochemical relapse after radical treatment for prostate cancer. Radiol Med 2008; 113: 895-904.
8) Oyen WJG, Witjes JA, Corstens FHM. Nuclear medicine techniques for diagnosis and therapy of prostate carcinoma. Eur Urol 2001; 40: 294-299.
9) Brush JP. Positron emission tomography in urological malignancies. Curr Opin Urol 2001; 11: 175-179.
10) Vees H, Bucheggert F, Albrecht S, Khan H, Husarik D, Zaidit H, Soloviev D, Hany TF, Mirabell R. 18F-choline and/or 11C-acetate PET: detection of residual or progressive subclinical disease at very low PSA values (<1 ng/mL) after radical prostatectomy. BJU Internat 2007; 99: 1415-1420.
11) Picchio M, Landoni C, Messa C, Gianolli L, Matarrese M, De Cobelli F, Maschio AD, Fazio F. Positive 11C-choline and negative 18F-FDG with PET in recurrence of prostate cancer. AJR 2002; 179: 482-484.
12) Rinnab L, Blumstein NM, Mottaghy FM, Hautmann RE, Küfer R, Hohl K, Reske SN. 11-Choline PET/CT and transrectal ultrasonography for staging localized prostate cancer. BJU Internat 2007; 99: 1421-1426.
13) Hain SF, Maisey MN. Positron emission tomography for urological tumours. BJU Internat 2003; 92: 159-164.
14) Toth G, Lengyel Z, Balkay L, Salah MA, Tron L, Toth C. Detection of prostate cancer with 11C-methionine PET. J Urol 2005; 173: 66-69.
15) Giorgetti A, Rufini V, Calcagni ML, Baum R. Imaging of neuroendocrine tumors. Semin Nucl Med 2006; 36: 228-247.
16) Grassi R, Cavaliere C, Cozzolino S, Mansi L, Cirillo S, Tedeschi G, Franchi R, Russo P, Cornacchia S, Rotondo A. Small animal imaging facilities: new perspectives for the radiologist. Radiol Med 2009; 114: 152-167.
17) Kong FL, Yang DJ. Amino-acid transporter-targeted radiotracers for molecular imaging in oncology. Curr Med Chem 2012; 19: 3271-3281.

18) Ellis RJ, Zhou EH, Fu P, Kaminsky DA, Sodee DB, Faulhaber PF, Bodner D, Resnick MI. SPECT with Capromab pentetide plus CT image set co-registration indipendently predicts biochemical failure. Urol 2008; 179: 1768-1774.

19) Kahn D, Williams RD, Haseman MK, Reed NL, Miller SJ, Gerstbrein J. Radioimmunoscintigraphy with In-111-labeled Capromab pentetide predicts prostate cancer response to salvage radiotherapy after failed radical prostatectomy. J Clin Oncol 1998; 16: 284-289.

20) Thomas CT, Bradshaw PT, Pollock BH, Montie JE, Taylor JMG, Thames HD, McLaughlin PW, DeBiase DA, Hussey DH, Wahl RL. ^{111}In-Capromab radioimmunoscintigraphy and prognosis for durable biochemical response to salvage radiation therapy in men after failed prostatectomy. J Clin Oncol 2003; 21: 1715-1721.

21) Golman K, Olsson LE, Axelesson O, Mansson S, Karlsson M, Petersson JS. Molecular imaging using hyperpolarized 13C. Br J Radiol 2003; 76: S118-127.

22) Albers MJ, Bok R, Chen AP, Cunningham CH, Zierhut ML, Zhang VY, Kohler SJ. Tropp J, Hurd RE, Yen YF, Nelson SJ, Vigneron DB, Kurhanewi CZ. Hyperpolarized 13C-lactate, pyruvate and alanine: noninvasive biomarkers for prostate cancer detection and grading. Cancer Res 2008; 68: 8607-8615.

23) Gallagher FA, Kettuhen MI, Day SE, Hu DE, Ardenkjaer-Larsen JH, Zandt R, Jensen PR, Karlsson M, Golman K, Lerche MH, Brindle KM. Magnetic resonance imaging of pH in vivo using hyperpolarized 13C-labelled bicarbonate. Nature 2008; 453: 940-943.

24) Casciani E, Polettini E, Bertini L, Emiliozzi P, Amini M, Pansadoro V, Gualdi GF. Prostate cancer: evalutation with endorectal MR imaging and 3D-proton spectroscopic imaging. Radiol Med 2004; 108: 530-541.

25) Polasek M, Fuchs BC, Uppal R, Schühle DT, Alford JK, Loving GS, Yamada S, Wei L, Lauwers GY, Guimaraes AR, Tanabe KK, Caravan P. Molecular MR imaging of liver fibrosis: a feasibility study using rat and mouse models. J Hepatol 2012; 57: 549-555.

26) Terreno E, Delli Castelli D, Cabella C, Dastrù W, Sanino A, Stancanello J, Tei L, Aime S. Paramagnetic liposomes as innovative contrast agents for MR molecular imaging applications. Chem Biodivers 2008; 5: 1901-1912.

27) Frascione D, Diwoky C, Almer G, Opriessnig P, Vonach C, Gradauer K, Lettinger G, Mangge H, Stolberger R, Prass R. Ultrasmall superparamagnetic iron-oxide (USPIO)-based liposomes as MR probes. Int J Nanomedicine 2012; 7: 2349-2359.

28) Terreno E, Dastrù E, Delli Castelli D, Gianolio E, Geninatti Crich S, Longo D, Aime S. Advances in metal-based probes for MR molecular imaging applications. Curr Med Chem 2010; 17: 3684-3700.

29) Onozato ML, Andrews PM, Li Q, Jiang J, Cable A, Chen Y. Optical coherence tomography of human kidney. J Urol 2010; 183: 2090-2094.

30) Abbandonato G, Signore G, Nifosì R, Voliani V, Bizzarri R, Beltram F. Cis-trans photoisomerization properties of GFP chromophore analogs. Eur Biophys J 2011; 40: 1205-1214.

31) Chen K, Xie J, Chen X. RDG-human serum albumin conjugates as efficient tumor targeting probes. Mol Imaging 2009; 8: 65-73.

32) Ohnishi S, Lomnes SJ, Laurence RG, Gagbashian A, Mariani G, Frangioni JV. Organic alternatives to quantum dots for intraoperative near-infrared fluorescent sentinel lymph node mapping. Mol Imaging 2005; 4: 172-181.

33) Kwon S, Ke S, Houston JP, Wang W, Wu Q, Li C, Sevick-Muraca EM. Imaging dose-dependent pharmacokinetics of RDG-fluorescent dye conjugate targeted to $\alpha V\beta 3$ receptor expressed in Kaposi's sarcoma. Mol Imaging 2005; 4: 75-87.

34) Kovar JL, Volcheck W, Sevick-Muraca EM, Simpson MA, Olive DM. Characterization and performance of near-infrared 2-deoxyglucose optical imaging agent for mouse cancer models. Anal Biochem 2009; 384: 254-262.

35) Adams KE, Ke S, Kwon S, Liang F, Fan Z, Lu Y, Hirschi K, Mawad ME, Barry MA, Sevick-Muraca EM. Comparison of visible and near-infrared wavelenght-excitable fluorescent dyes for molecular imaging of cancer. J Biomed Opt 2007; 12: 024017.

36) Laxman B, Hall DE, Bhojani MS, Hamstra DA, Chenevert TL, Ross BD, Rehemtulla A. Noninvasive real-time imaging of apoptosis. PNAS 2002; 99: 16551-16555.

37) Wang S, Li W, Zue Z, Lu Y, Narsinh K, Fan W, Li X, Bu Q, Wang F, Liang J, Wu K, Cao F. Molecular imaging of p53 signal pathway in lung cancer cell cycle arrest induced by cisplatin. Mol Carcinog 2012; (in press)

38) Fillat C, Carrio M, Cascente A, Sangro B. Suicide gene therapy mediated by H.S.V. thymidine kinase gene/ganciclovir system: fifteen years of application. Curr Gene Ther 2003; 3: 13-26.

39) Russell SJ, Peng K-W. Gene therapy. Mayo Clin Proc 2003; 78: 1370-1383.

40) Weissleder R. Molecular imaging: exploring the next frontier. Radiology 1999; 212: 609-614.

41) Bangma CH, Mongiat P, Kraaij R, Schenk-Braat E. Gene therapy in urology: strategies to translate theory into practice. BJU Internat 2005; 96: 1163-1170.

42) Malmberg J, Sandström M, Wester K, Tolmachev V, Orlova A. Comparative biodistribution of imaging agents for in vivo molecular profiling of disseminate prostate cancer in mice bearing prostate cancer xenografts. Nucl Med Biol 2011; 38: 1093-1102.

43) Kataoka M, Yamaguchi Y, Moriya Y, Sawada N, Yasuno H, Kondoh K, Evans DB, Mori K, Hayashi S. Antitumor activity of chemoendocrine therapy in premenopausal and postmenopausal models with human breast cancer xenografts. Oncol Rep 2012; 27: 303-310.

44) Zhou W, Zhu H, Chen W, Hu X, Pang X, Zhang X, Huang X, Fang B, He C. Treatment of patient tumor-derived colon cancer xenografts by a TRAIL gene-armed oncolytic adenovirus. Cancer Gene Ther 2011; 18: 336-345.

45) Serwer LP, Noble CO, Michaud K, Drummond DC, Kirpotin DB, Ozawa T, Prados MD, Park JW, James CD. Investigation of intravenous delivery of nanoliposomal topotecan for activity against orthotopic gliobastoma xenografts. Neuro Oncol 2011; 13: 1288-1295.

46) Zhang S. Fabrication of novel biomaterial through molecular self-assembly. Nature Biotechnol 2009; 21: 1171-1178.

47) Hutmacher DW, Loessner D, Rizzi S, Kaplan DL, Mooney DJ, Clements JA. Can tissue engineering concepts advance tumor biology research? Trends Biotechnol 2010; 28: 125-133.

48) Schanz J, Pusch J, Hansmann J, Walles H. Vascularized human tissue models: a new approach for the refinement of biomedical research. J Biotechnol 2010; 148: 56-63.

49) Ferrari M. Cancer nanotechnology: opportunities and challenges. Nat Rev Cancer 2005; 5: 161-171.

50) Singh AK, Hahn MA, Gutwein LG, Rule MC, Knapik JA, Moudgil BM, Grobmyer SR, Brown SC. Multidye theranostic nanoparticle platform for bioimaging and cancer therapy. Int J Nanomedicine 2012; 7: 2739-2750.

51) Yaku H, Fujimoto T, Murashima T. Miyoshi D, Sugimoto N. Phthalocyanines: a new class of G-quadruplex-ligands with many potential applications. Chem Commun 2012; 48: 6203-6216.

52) Yang K, Hu L, Ma X, Ye S, Cheng L, Shi X. Li C, Li Y, Liu Z. Multimodal imaging-guided photothermal therapy using functionalized graphene nanosheets anchored with magnetic nanoparticles. Adv Mater 2012; 24: 1868-1872.

53) Yang X, Liu X, Liu Z, Pu F, Ren J, Qu X. Near-Infrared light-triggered, targeted drug delivery to cancer cells by aptamer gated nanovehicles. Adv Mater 2012; 24: 2890-2895.

54) Ren T, Liu Q, Lu H, Liu H, Zhang X, Du J. Multifunctional polymer vesicles for ultrasensitive MRI and drug delivery. J Mater Chem 2012; 22: 12329-12338.

55) Baum RP, Kulkarni HR. Theranostic: from molecular imaging using Ga-68 labeled tracers and PET/CT to personalized radionuclide therapy. The Bad Berka experience. Theranostics 2012; 2: 437-447.

56) Melancon MP, Stafford RJ, Li C. Challenges to effective cancer nanotheranostics. J Control Release 2012; 164: 177-182.

57) Kumar R, Kulkarni A, Nagesha DK, Sridhar S. In vitro evaluation of theranostic polymer micelles for imaging and drug delivery in cancer. Theranostics 2012; 2: 714-722.

58) Mayor JL, Meade TJ. Bioresponsive, cell-penetrating and multimeric MR contrast agents. Acc Chem Res 2009; 42: 893-903.

59) Tempani CM, McNeil BJ. Advance in biomedical imaging. JAMA 2001; 285: 562-567.

60) Miller AD. Towards technologies for nucleic acid therapy. Tumori 2008; 94: 234-245.

61) Bu L, Xie J, Chen K, Huang J, Aguilar ZP, Wang A, Sun KW, Chua MS, So S, Cheng Z, Eden HS, Shen B, Chen X. Assessment and comparison of magnetic nanoparticles as MRI contrast agents in a rodent model of human hepatocellular carcinoma. Contrast Media Mol Imaging 2012; 7: 363-372.

62) Hamad-Schifferli K, Schwartz J. Santos A, Zhang S, Jacobson J. Remote electronic control of DNA hybridization through inductive coupling to an attached metal nanocrystal antenna. Nature 2002; 415: 152-155.

63) Yang J. Mens-based probe recording technology. J Nanosci Nanotechnol 2007; 7: 181-192.

64) Jokerst JV, Gambhir SS. Molecular imaging and theranostic nanoparticles. Acc Chem Res 2011; 44: 1050-1060.

65) Li Y, Li Z, Wang X, Liu F, Cheng Y, Zhang B, Shi D. *In vivo* cancer targeting and imaging-guided surgery with NIR-emitting quantum dot bioconjugates. Theranostics 2012; 2: 769-776.